deletion of the gene for said indigenous extracellular protease transformed with an expression cassette providing for expression of a mutated high alkaline protease exhibiting altered protease activity in said host, whereby said mutated high alkaline protease is produced.

Remarks

A list of the pending claims is attached for the convenience of the Examiner.

The Invention

The claimed invention is directed to methods and compositions for preparation of mutant high alkaline proteases and mutant alkalophilic *Bacillus* strains which produce only the mutant high alkaline protease and not the corresponding indigenous protease. Also claimed are a detergent composition comprising as an active ingredient one or more high alkaline proteases in a detergent composition or a laundry process.

The Pending Claims

Claims 4-7, 9, 10-17, 19, 23-26 are pending in the application. Claims 23, 4-7, 9-11, and 26 are directed to methods for production of a mutant high alkaline protease; Claims 12-13 are directed to a method of obtaining an alkalophilic *Bacillus* strain having a reduced extracellular alkaline protease level; Claims 14-16 are directed to an alkalophilic *Bacillus* strain producing a mutant high alkaline protease; Claim 17 is directed to mutant high alkaline protease; Claim 19 is directed to a detergent composition comprising as an active ingredient high alkaline protease. Claim 24 is directed to a method of preparing a detergent composition comprising high alkaline protease as active ingredient. Claim 25 is directed to a method of processing laundry with the claimed detergent composition.

The Final Office Action

The Examiner rejects claims 4-7, 9-17, 19, and 23-26, under 35 U.S.C. §112 second paragraph, asserting that the disclosure is enabling only for claims limited to methods of producing an alkalophilic asporogenic Bacillus novo species PB92 of minimal indigenous extracellular protease level, transformed with a mutated B. novo PB92 alkaline protease.

The Examiner rejects claims 4-17, 19, 23-26 under 35 U.S.C. §103, asserting that the claims are unpatentable over Fahnestock et al. and Estell et al., in view of TeNijenhuis and Suggs et al.

The Amendments

Claims 9, 14, 19, 23-26 have been amended to include a mutant alkaline protease exhibiting altered protease activity. Support for this limitation can be found in the specification pg. 29, lines 14-16. Claim 17 has been amended to include a mutant high alkaline protease differing in at least one amino acid from the indigenous high alkaline protease wherein said mutant maintains capacity to degrade substrate. Support for the limitation can be found on pg. 13, 10-16.

35 U.S.C. §112

Claims 4-7, 9-17, 19, 23-26 stand rejected on the basis that the disclosure is enabling only for claims limited to methods of producing an alkalophilic asporogenic Bacillus novo species PB92 of minimal indigenous extracellular protease level, transformed with a mutated B. novo PB92 alkaline protease. Applicant traverses this rejection.

The Examiner has maintained this rejection in the past three office actions. In the first Response filed 4/12/92, the Applicant pointed out that the claims are not directed to methods of producing a strain of Bacilli, but as amended, are directed to methods for producing mutant high alkaline protease substantially free of indigenous extracellular protease. Methods of producing mutant high alkaline protease are described beginning on page 12,

line 22. In the second Response filed 12/15/92, the Applicant pointed out that a key inventive concept of the instant application lies in the discovery by the Applicant that Bacillus strains could be made that have a reduced protease level by deleting an indigenous protease gene. Such altered Bacillus strains possess the surprising quality that they are particularly suitable for the production of a mutant protease introduced by transformation with a mutant gene. As the Applicant noted, the invention was exemplified and preferably carried out with alkalophilic Bacilli, particularly B. novo species PB92 in which the indigenous high alkaline protease gene has been deleted and then the mutated Bacilli transformed with a mutant high alkaline protease gene. The invention was exemplified using a gene for the high alkaline protease of Bacillus novo species PB92. Applicant has urged that one of ordinary skill in the art would recognize that the invention could be practiced with any alkalophilic Bacillus. The Examiner has considered these arguments, but deemed them all unpersuasive.

In this third Response, the Applicant respectfully points out that the disclosure teaches more than methods of producing an alkalophilic asporogenic Bacillus novo species PB92 of minimal indigenous extracellular protease level, transformed with a mutated B. novo PB92 alkaline protease, as alleged by the Examiner. The specification teaches that several types of Bacillus strains and alkaline proteases may be used with the invention. For example, the specification refers to the Bacillus novo species PB92 strain as an example of an alkalophilic Bacillus host strain and discloses other alkalophilic Bacillus strains that can be used for protease production (pg. 12 lines 8-21). DNA Transformation protocols needed for these strains have also been disclosed (pg. 14, lines 23-25). The specification also describes other mutant high alkaline proteases (sentence bridging pp. 12 and 13). The claimed methods find use in the production of mutant serine proteases using protease negative strains of alkalophilic Bacillus (pg. 4, lines 32-35; sentence bridging pp. 6-7). Examples of alkalophilic protease mutants

cloned by applicants include M216Q, M216S, S160D, N212D as provided on page 20 of the specification.

The specification need not provide a detailed disclosure regarding all the possible Bacillus strains and proteases that could be used in the instant invention, since references to the art have been provided. The Examiner is respectfully reminded that the specification need not disclose and preferably omits that which is well known in the prior art, *Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986)*. Taken together, the specification has disclosed methods of using several Bacillus strains and proteases to produce modified Bacilli which produce mutant protease in the absence of indigenous protease(s).

The applicant maintains that it would not require inventive skill or undue experimentation to construct a variety of mutant protease-producing Bacillus strains. The Examiner is respectfully reminded that it is improper to require the applicant to limit claims only to working examples provided in the specification, since it would unnecessarily deny the Applicant coverage of embodiments to which he is entitled. As stated by the *Goffe* court:

> "To demand that the first to disclose shall limit his claims to what he has found will work or to materials which meet guidelines specified for "preferred" materials in a process such as the case herein involved would not serve the constitutional purpose of promoting progress in the useful arts."
> *In re Goffe*, 542 F.2d. 564, 191 U.S.P.Q. 426 (1976).

The Examiner asserts that the claims are not properly enabled for the recitation of the phrases "mutant high alkaline protease" and "differing in at least one amino acid from a wild-type high alkaline protease". The Examiner is concerned that "one of ordinary skill in the art would not be able to determine what type of mutation, how many, at what amino acid, etc., including all variations possible... [and] one skilled in the act could not prophetically predict the outcome of any mutation upon the gene.." The Applicant respectfully points out that knowing

the type of mutation induced in a particular protease gene or the effect of a mutation on protease function is not required in order to practice the instant invention. For example, the skilled artisan could easily determine the termini of a test protease gene in a plasmid by using standard restriction enzyme mapping techniques. Once the protease gene termini are determined, a restriction enzyme could be chosen that removes the test protease gene sequence from the plasmid, but leaves the termini within the plasmid. Appropriate starting vectors and routine methods for manipulating DNA fragments between plasmids are disclosed pp. 15-16. The applicant urges that these techniques are routine and require no inventive skill or undue experimentation. Once a plasmid is made that comprises the termini of the test protease gene, it can be used to transform a Bacillus host (i.e. PB92), resulting in integration and inactivation of the host protease gene (pp. 16-17). The original plasmid comprising the test protease gene can be mutated by digestion with another restriction enzyme and used to transform the mutant Bacillus host in order to produce mutant protease (pg. 25-27). Success in practicing the invention, whether molecular information is available or not, is evaluated by methods for selecting and characterizing protease negative strains as provided on pg. 17 and methods for evaluating protease activity as provided on pg. pg. 28 line 27 - pg. 29 line 45. *The Applicant stresses that a knowledge of protease gene structure before or after mutagenesis and the effect of gene mutation on protease activity is not essential to practice the instant invention.* The claims have been amended to include the limitation that a mutant high alkaline protease is evaluated not by any knowledge of gene structure, but simply by determining whether the mutant protease exhibits altered protease activity (pg. 29, lines 12-16) or maintains the capacity to degrade substrate (pg. 13, lines 10-13).

In view of the above amendments and remarks, Applicant respectfully submits that this rejection should be withdrawn.

35 U.S.C. §103

The Examiner rejects claims 4-17, 19, 23-26, asserting that they are unpatentable over Fahnestock et al. and Estell et al., in view of TeNijenhuis and Suggs et al. This rejection is respectfully traversed as follows.

Two key inventive concepts of the instant invention are that 1) mutant proteases can be produced efficiently by using improved Bacillus strains comprising a mutant protease gene, preferably a mutant protease gene lacking a complete coding region and 2) asporogenous Bacilli are surprisingly useful in obtaining high level expression of a mutant protease gene. In substantiating a §103 rejection, it is essential to compare the claims and the prior art in view of the entire inventive concept outlined above and without the benefit of hindsight afforded by the instant application. It is impermissible to dismiss any surprising or unexpectedly useful properties of the instant invention. Case law has repeatedly held that the above-mentioned method of substantiating a §103 rejection is correct (see *Perkin -Elmer Corp. v. Computervision Corp., 732 F.2d 888, 221 USPQ 669 (Fed. Cir. 1984); In re Fine, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); In re Wright, 848 F.2d 1216, 6 USPQ2d 1959 (Fed. Cir. 1988)*. Furthermore, there must be some teaching, suggestion or incentive supporting the combination of prior art against the claims (*ACS Hospital Systems, Inc. v. Montifiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)*.

Fahnestock et al. teaches Bacillus strains having reduced levels of protease that are produced by inactivating the endogenous protease with a partially homologous DNA sequence having an inactivating CAT sequence therein. The Examiner is incorrect to assert that because Fahnestock et al. use homologous recombination, this obviates the instant invention (instant office action pg. 3, lines 29-32). The genetic technique of homologous recombination is well known in the art and is not a unique contribution of the Fahnestock et al. patent. More importantly, there is no incentive, teaching or suggestion in Fahnestock et al. to improve their method of obtaining protease-

producing Bacillus by mutating the homologous protease gene without a CAT insert or to delete the entire protease gene, nor is there any incentive, teaching or suggestion to use an alkalophilic Bacillus as a starting strain.

Estell et al. teach a cloned subtilisin gene that has been modified at specific sites by a chemical mutagen to cause amino acid substitutions. Chemical mutagenesis, as taught by Estell et al., causes partial deletion of the subtilisin gene. The Examiner's statement that "[i]t would have been obvious to further delete the rest of the coding region, for the mere assurance of complete success of no protease activity" is grossly improper. The Examiner is clearly interpreting Estell et al. in light of the teachings of the instant application to substantiate an obviousness rejection. This approach is clearly prohibited by case law (supra). The Examiner's statement also dismisses a key inventive concept in the instant application, namely that mutation of the homologous protease gene, preferably complete deletion, will result in an improved Bacillus strain for protease production. Finally, there is no incentive, teaching or suggestion in Estell et al. to use alkalophilic Bacillus as a starting strain.

As stated in previous Responses, Suggs et al. only disclose a general description of how to perform mutagenesis using synthetic oligonucleotides. TeNijenhuis disclose detection of an alkaline protease in media containing B. novo species PB92. There is no teaching, suggestion or incentive in either reference, alone or in combination, to make improved Bacillus strains or use alkalophilic Bacilli.

The Examiner concludes the §103 rejection by suggesting that the limitations "alkalophilic Bacillus strain" and "asporogenic" are not patentably distinct from methods as cited above. The Examiner makes this remark despite the fact that the specification teaches problems known in the art when Bacilli have been used to produce proteases (pg. 1, line 31 - pg. 2, lines 1-19). The use of asporogenic Bacillus strains were believed to be especially unsatisfactory (pg. 2, line 10-14) and special methods

of transforming alkalophilic Bacillus are required (pg. 14, lines 23-25). The disclosure by Estell et al. is indicative of the knowledge in the art at the time the invention was made: "asporogenous [bacilli]... are unsatisfactory for the recombinant production of heterologous proteins because asporogenous mutants tend to lyse during earlier stages of their growth cycle.." (Estell et al. column 2, lines 27-33). The Applicant urges that Estell et al. teach away from using asporogenous bacilli for the production of proteases. Thus, the utility of alkalophilic Bacilli, especially asporogenous alkalophilic Bacilli, is surprising and unexpected and removes a key inventive concept of the instant invention. The Examiner's remark that asporogenous Bacilli can be produced by classic UV mutagenesis is irrelevant, because it ignores the surprising properties of asporogenous Bacilli in the instant invention.

Conclusion

It is respectfully submitted that this application is now in condition for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call to the undersigned at (415) 854-5277. Please charge any additional fees, or make any credits, to Deposit Account No. 06-1050.

Respectfully submitted,

Date: September 2, 1993

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PENDING CLAIMS
USSN 07/565,573

4. The method according to Claim 23, wherein said Bacillus strain is Bacillus novo species PB92 or a derivative thereof.

5. The method according to Claim 23, wherein said Bacillus strain is an asporogenic alkalophilic Bacillus strain.

6. The method according to Claim 23, wherein the gene encoding said indigenous protease has been deleted by homologous or illegitimate recombination.

7. The method according to Claim 23, wherein a plasmid comprises said expression cassette.

9. The method according to Claim 7, wherein said mutant high alkaline protease exhibiting altered protease activity is obtained from Bacillus novo species PB92.

10. The method according to Claim 23, wherein at least one copy of said expression cassette is integrated into the genome of said host.

11. The method according to Claim 10, wherein said host further contains at least one copy of a plasmid comprising said expression cassette.

12. A method of obtaining an alkalophilic Bacillus strain having no detectable extracellular high alkaline protease, said method comprising:

transforming an alkalophilic Bacillus strain with a cloning vector comprising the 5′ and the 3′ flanking regions but not the coding region of a gene coding for the high alkaline

protease and wherein a sufficient amount of said flanking regions is present to provide for homologous recombination with an indigenous gene coding for the high alkaline protease whereby transformants are obtained;

growing said transformants under conditions whereby the replication function encoded by said vector is inactivated; and

isolating transformants identified as having no detectable extracellular high alkaline protease.

13. The method according to Claim 12, wherein said alkalophilic Bacillus strain is Bacillus novo species PB92 or a derivative thereof.

14. An alkalophilic Bacillus strain producing a mutant high alkaline protease exhibiting altered protease activity and substantially free of expression product of an indigenous extracellular alkaline protease gene, wherein said strain has been obtained by transforming an alkalophilic Bacillus strain having no detectable indigenous extracellular high alkaline protease obtained by the method according to Claim 12 or 13 with a plasmid expression vector comprising the mutant high alkaline protease gene.

15. The Bacillus strain according to Claim 14, wherein aid mutant alkalophilic Bacillus strain is a mutant of Bacillus novo species PB92 or a derivative thereof.

16. The Bacillus strain according to Claim 15, wherein said indigenous gene has been deleted by homologous or illegitimate recombination.

17. A mutant high alkaline protease produced according to the method of Claim 23 and characterized as (1) substantially free from contamination with an indigenous extracellular high alkaline protease, and (2) differing in at least one amino acid from the indigenous high alkaline protease sufficient to maintain capacity to degrade substrate.

19. A detergent composition comprising as an active ingredient one or more high alkaline protease exhibiting altered protease activity prepared according to the method of Claim 23.

23. A method for production of a mutated high alkaline protease exhibiting altered protease activity and substantially free of indigenous extracellular high alkaline protease, said method comprising:

growing an alkalophilic Bacillus strain host having no detectable indigenous extracellular protease as a result of deletion of the gene for indigenous extracellular protease transformed with an expression cassette providing for expression of a said mutant high alkaline protease in said host, whereby said mutant high alkaline protease is produced.

24. A method for preparing a detergent composition, which comprises the step of combining a detergent composition with, as an active ingredient, one or more of a high alkaline protease exhibiting altered protease activity prepared according to the method of Claim 23.

25. A method for processing laundry, which comprises the step of contacting said laundry with a detergent composition comprising as an active ingredient one or more of a high alkaline

protease exhibiting altered protease activity prepared according to the method of Claim 23.

26. A method for production of a mutated high alkaline protease exhibiting altered protease activity substantially free of indigenous extracellular protease, said method comprising:

growing an asporogenous Bacillus strain host having a reduced indigenous extracellular protease level as a result of deletion of the gene for said indigenous extracellular protease transformed with an expression cassette providing for expression of a mutated high alkaline protease exhibiting altered protease activity in said host, whereby said mutated high alkaline protease is produced.